Hypothalamic neuronal activity and intermediary metabolism in *ob/ob* mice

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Introduction: Obesity is a pandemic syndrome, associated with the most prevalent and mortal pathologies in developed countries (Rajala and Scherer, 2003). This pathology results from an imbalance in the control of food intake and energy homeostasis as regulated by a complex network of peripheral and intra-hypothalamic signalling systems (Schwartz et al., 2000). On this basis, the investigation of neuroglial coupling mechanisms and energy metabolism in the hypothalamus is essential to the understanding and integration of the pathways regulating whole body energy homeostasis. Leptin, a cytokine hormone secreted by the adipose tissue, plays an important role in the physiologic regulation of appetite and energy balance by reducing body weight via interaction with hypothalamic neurons (Zhang et al., 1994). In this study, we compared the hypothalamic neuronal activation and intermediary metabolism between wild type (*wt*) and leptin-deficient (*ob/ob*) mice through the administration of [1-^{13}C]glucose and applying a regional ^{13}C HR MAS approach.

Methods: C57BL/6 (n=6 per condition) and C57BL/6J *ob/ob* (n=6 per condition) male mice (8-10 weeks old), receiving drinking water *ad libitum*, were conditioned by feeding (normal chow diet) or fasting (food removed overnight before the experiment) conditions. All mice received an i.p. injection of [1-^{13}C]glucose (20 µmol/g body weight) and, 15 minutes later, cerebral metabolism was arrested using a high-power (5 kW) focused microwave fixation system. The brain was dissected and divided in two areas, hypothalamus and remaining brain. Samples were analyzed in an 11.7 T (125.13 MHz) Bruker AVANCE WB NMR spectometer (4 kHz spinning, 4 ºC). All ^{13}C HR-MAS resonance areas were normalized to the *myo*-inositol C1, C3 resonance area, to account for differences in tissue content within the different samples. A two-way ANOVA with Bonferroni's post-test was applied to compare differences in the metabolites contents between fed and fasted conditions in both mice models. Comparisons with $p<0.05$ were considered statistically significant.

Results: ^{13}C HR MAS spectra obtained from the biopsies allowed the study of the mice cerebral metabolism in hypothalamus and remaining brain. We investigated the incorporation of [1-^{13}C]glucose into lactate (Lac) C3, glutamate (Glu) C4, glutamine (Gln) C4 and GABA C2 resonances, relative to the natural abundance *myo*-inositol C1,C3 resonance, as represented in Figure 1 for both *wt* and *ob/ob* mice in fed (left panel) and fasted (right panel) conditions. In the fed state *ob/ob* mice show higher Glu C4 and Gln C4 concentrations compared to *wt* mice (A). Fasting resulted in increased Lac C3 and GABA C2 concentrations in the hypothalamus of both mice (B). Additionally, GABA C2 concentrations increased more in *ob/ob* mice (A). The Glu and Gln C4 concentrations remained higher for the *ob/ob* compared to *wt* (A). Fasted mice of both types have higher Lac C3 concentration in the hypothalamus than fed mice ($p<0.05$, statistics not showed in the graphs).

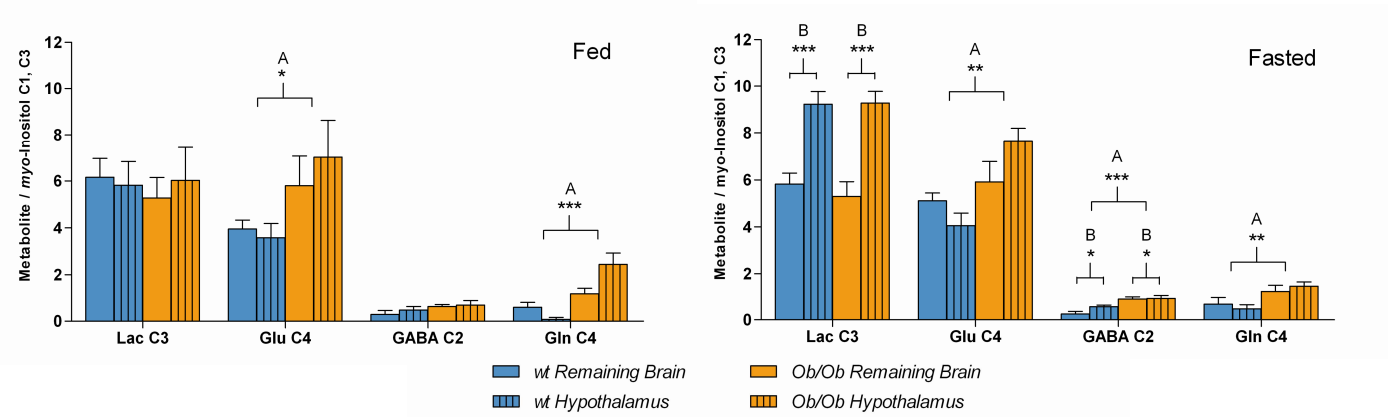

Figure 1 – ^{13}C incorporation from (1-^{13}C) glucose into Lac C3, Glu C4, GABA C2 and Gln C4 in the hypothalamus and remaining brain of *wt* and *ob/ob* mice in fed (left panel) and fasted (right panel) conditions. Metabolites are normalized to *myo*-inositol C1, C3 resonance. Data were analyzed by two-way ANOVA, A represents the comparison between animal type and B between brain region. Values represent mean ± SEM.

Conclusions: Increased hypothalamic content of Lac C3 and GABA C2 under fasting conditions occurred in both *wt* and *ob/ob* mice showing that this is not a leptin-dependent effect. The higher concentration of these metabolites in the hypothalamus could result from an increased astrocyte-to-neuron lactate shuttle activity and either elevated GABA synthesis, and/or decreased GABA degradation. The higher concentrations of Glu and Gln C4 in *ob/ob* mice reveal an increased glutamate/glutamine cycle activity and consequently an increased glutamatergic neurotransmission. Taken together, these results suggest that leptin signalling in the hypothalamus may involve, in addition to the well known neuropeptide signalling systems of the hypothalamus, increased glutamatergic neurotransmission.

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